Diallel analysis of sugar composition of 10 vegetable soybean lines

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Abstract

Few studies have evaluated vegetable soybean for sugar content at the green pod stage. Information on combining ability and type of gene action that governs inheritance of seed traits can help breeders to select suitable parents and devise an appropriate breeding strategy. Ten vegetable soybean accessions were crossed in a complete diallel mating design. Parent lines and F_2 and F_3 progenies were evaluated for two nutritional components. In this study, both general and specific combining ability and reciprocal effects were significant for sucrose and total sugar. Cultivars 'Kanrich', 'Pella', 'Verde' and V81-1603 had good general combining ability for high sucrose. In general, high sucrose content was observed in progeny of the early maturity group genotypes 'Kanrich', 'Pella' and 'Verde'. The best combiners for high total sugar content were 'Verde', V81-1603 and PI 399055. These genotypes could serve as genetic sources in a vegetable soybean breeding programme.

Key words: *Glycine max* — additive variance — vegetable soybean — edamame — combining ability — sugar — sucrose

The USDA Dietary Guidelines encourage the consumption of three to five servings of vegetables daily (USDA 1995). Introducing green soybean as a vegetable will add a new food item to the American diet. Edamame vegetable soybean is a speciality product that is harvested when seeds are still immature in the R6–R7 growth stage (Fehr et al. 1971, Mebrahtu et al. 2005, 2007). Edamame, a Japanese word for 'green vegetable soybean', is a traditional food of Japan and China (Yinbo et al. 1997). In Asia, farmers harvest fresh green pods when the pods are fully filled and just prior to turning yellow (Shanmugasundaram et al. 1991). The fresh or frozen vegetable soybean can be cooked like sweet pea or lima bean, either stir-fried or added to stews and soups. In Japan, edamame is commonly used as a snack. Boiled salted pods should be blemish-free and bright green (Carter and Shanmugasundaram 1993). Masuda (1991) compared vegetable soybean quality with that of green peas and reported that the nearly 50% greater protein content of the fresh green soybean makes them particularly desirable for health-conscious people seeking low-fat, high-protein snacks (Brar and Carter 1993).

The flavours most desired in beans are said to be sweetness and nuttiness and absence of 'beany taste' (Young et al. 2000, Mohamed and Rao 2004). A desirable vegetable soybean should have very large seed size (> 20 g/100 seeds), high sugar levels, a smooth texture and possess dry weight sucrose content > 10% from mid-pod development until maturity.

In the USA, speciality soybean is a niche-market commodity. The limited consumer base and lack of suitable cultivars are the factors limiting domestic vegetable soybean production. There is a need to introduce, evaluate and characterize vegetable soybean cultivars of domestic and Asian origin for suitability for cultivation in the USA. Development of improved vegetable soybean cultivars offers potential for expanding the domestic and international soybean market.

The diallel analysis as presented by Griffing (1956) is useful for studying genetic variation of specific traits and identifying crosses likely to produce superior segregants. Genetic information on desirable traits is needed for an efficient breeding plan for vegetable soybean. The objectives of this study were to: (i) elucidate the nature and magnitude of the different types of gene action involved; (ii) obtain estimates of general and specific combining abilities and (iii) assess the breeding value of parent lines.

Materials and Methods

The 10 vegetable soybean (Glycine max [L.] Merr.) genotypes used were: 'Kanrich' (P1), 'Pella' (P2) and 'Verde' (P3) from Maturity group (MG) III; 'Tomahomare' (P4), PI 506852 (P5) and PI 379621 (P6) from MG IV; V81-1603 (P7) and PI 399055 (P8) from MG V; and VS95-50 (P9) and 'Late Giant' (P10) from MG VI. These were chosen on the basis of green pod yield, nutritional values and panel taste test (Young et al. 1997, Mebrahtu and Hallowell 1998). A complete diallel set of 90 crosses, including reciprocals, was made during the summer of 1997. The 90 F_1 hybrids and their corresponding 10 parents were grown in 18 cm plastic pots in the greenhouse to generate F_2s. The F_3 progenies were harvested from approximately 10 F_2 field-grown plants. The genetic materials comprising 100 entries, including 10 parents, their 45 F_2 hybrids and 45 F_2 reciprocals were planted in 1998 for field evaluation. The parents and F_3s were planted in the 1999 growing season for field evaluation. The experimental design was a randomized complete block design (RCBD) with four replications at one location each year. Experimental units were single-row plots 4.0×0.75 m. Evaluations were conducted at the Randolph Research Farm of Virginia State University, Petersburg, Virginia on Abell sandy loam (aquatic Hapridults, fine loamy mixed, thermic). Genotypes were evaluated at their green-bean growth stages (R6–R7) by harvesting 25 plants at random from each plot. The decision to harvest was made when the pods were green and plump and the seeds were green and nearly full sized, approximately 35–40 days after 50% of the plants had flowered. The harvested materials were immediately put into plastic bags and brought to the laboratory. Pods were removed manually in the laboratory.

Sugar analysis: Pod samples were taken at random from each harvested plot. The beans were shelled from pods by hand, freeze-dried, ground and analysed for sugars (glucose, fructose, sucrose,

raffinose and stachyose) via a high performance liquid chromatography (HPLC) system as described by Kennedy et al. (1985). Sucrose content was expressed as mg/g and the total sugar was the summation of all individual sugars (glucose, fructose, sucrose, raffinose and stachyose) and expressed as mg/g.

Data analysis: The analysis of variance procedure was applied to the F_2 and F_3 data using the method appropriate for the RCBD. As significant genotypic differences were observed, the data were also analysed further using AGROBASE (Agronomix 2000) for general combining ability (GCA), specific combining ability (SCA) and using Griffing (1956) Method 1, Model I for reciprocal (RECIP) differences. The relative importance of GCA and SCA effects is calculated as GCA/SCA means squares. The Spearman Rank correlation was computed between the GCA effects and overall means for the nutritional values of each of the F_2 and F_3 analyses. The means were separated via least significant differences (LSD) at the 5% probability level as described by Steel and Torrie (1980).

Results

The analysis combined over years indicated highly significant ($P = 0.01$) year, genotypic and genotype × year interaction (GYI) variance effects for sucrose and total sugar data (Table 1). The sum of squares for each seed trait, expressed as a percentage, illustrated the relative contribution of each source to the total sum of squares. These values are presented in Table 1. The year effect contributed proportionally less than the genotype, and GYI, but was higher than the replication within year [Rep (yr)] and pooled error sum of squares. The proportional contributions of genotype sum of squares for all seed traits were higher than the GYI contributions.

Highly significant differences ($P = 0.01$) were found for sucrose and total sugar among genotypes. These permitted further analysis of combining abilities.

Sucrose

The significant GCA and SCA indicated the importance of both additive and non-additive gene action for the inheritance of sucrose (Table 1). The GCA/SCA ratios for sucrose were 2.23 for F_2 and 5.46 for F_3 analysis, indicating that the prediction of parental performance can be made on the F_2 GCA effects alone. The sucrose mean ranged from 45.7 mg/g for PI 506852 to 90.2 mg/g for 'Kanrich', with an overall mean of 74.4 mg/g (Table 2). 'Kanrich', 'Pella', PI 399055 and VS95-50 were the parents with a significant higher sucrose mean than the overall mean.

The sucrose mean was lower in the F_3 analysis from samples collected in 1999 than in the F_2 analysis from

Source of variation	Parents – Sucrose		Parents – Total sugar	
	Mean square	% contribution[1]	Mean square	% contribution[1]
Year	1166.27**	6.65	2790.88**	12.05
Rep (Year)	156.85	0.89	257.27	1.11
Parent (P)	11550.27**	65.85	12744.91**	55.04
P × Year	3847.08**	21.93	6084.78**	26.28
Pooled error	818.73	4.67	1275.87	5.51

	Mean squares			
	F_2 - Sucrose	F_3 - Sucrose	F_2 - Total sugar	F_3 – Total sugar
GCA	501.98**	857.42**	799.05**	1130.46**
SCA	225.38**	157.09**	289.66**	182.97**
RECIP	372.34**	158.43**	520.12**	203.62**
Error	8.47	9.45	20.00	10.98
CV %	6.80	9.02	7.70	7.18
GCA/SCA ratio	2.23	5.46	2.76	6.18

GCA, general combining ability; SCA, specific combining ability, RECIP, reciprocal.
**Significantly different at 0.01 probability level.
[1]Relative percentage contribution of each sources of variation to total variance.

Table 1: Analysis of variances combined over years for sucrose and total sugar content of 10-parental vegetable soybean lines, and complete diallel analysis (general and specific combining abilities, and reciprocal) of parents, F_2 progenies planted in 1998 and F_3 progenies planted in 1999

	Sucrose				Total sugar			
	Mean (mg/g)		GCA		Mean (mg/g)		GCA	
Parents	F_2	F_3	F_2	F_3	F_2	F_3	F_2	F_3
'Kanrich' (P1)	90.2	90.2	0.8	10.9	115.1	115.1	−0.3	13.7
'Pella' (P2)	80.0	80.0	2.4	9.4	100.6	100.5	−4.2	10.9
'Verde' (P3)	74.4	72.6	1.9	3.3	99.5	95.4	2.6	2.7
'Tomahomare' (P4)	58.5	42.4	−7.2	−3.3	82.7	63.8	−9.2	−3.0
PI 506852 (P5)	45.7	60.6	2.1	−4.9	67.6	77.9	1.6	−5.5
PI 379621 (P6)	46.8	46.8	−3.8	−7.2	71.3	71.3	−4.4	−6.1
V81-1603 (P7)	73.7	74.5	7.0	4.9	102.2	96.8	9.9	4.4
PI 399055 (P8)	88.2	44.4	5.6	−3.9	124.0	64.3	8.1	−4.1
VS95-50 (P9)	74.4	47.7	3.4	−3.8	104.1	66.8	3.2	−7.1
'Late Giant' (P10)	52.2	42.8	−7.4	−5.1	83.4	61.9	−7.7	−5.8

Sucrose: Mean – F_2, 69.0; F_3, 60.0. LSD$_{(0.05)}$ – F_2, 5.3; F_3, 7.9. GCA – S.E. – F_2 G_i, ± 0.6; F_3 G_i, ± 0.7.
Total sugar: Mean – F_2, 95.0; F_3, 81.4. LSD$_{(0.05)}$ – F_2, 7.06; F_3, 9.6. GCA – S.E. – F_2 G_i, ± 1.0; F_3 G_i, ± 0.7.

Table 2: Mean seed sucrose and total sugar contents (mg/g) and general combining ability effects of ten parental vegetable soybean lines in F_2 and F_3 generations. F_2 progenies were planted in 1998 and F_3 progenies were planted in 1999

samples collected in 1998. In the F_3, the overall mean value was 59.0 mg/g compared with 74.5 mg/g for the F_2. In the F_3, the parents with significantly higher means than the overall mean were 'Kanrich', 'Pella', 'Verde' and V81-1603. The parent lines with significantly consistent higher sucrose content in both F_2 and F_3 were 'Kanrich' and 'Pella'. These parents are large-seeded vegetable types (Mebrahtu et al. 1991). The diversity for sucrose content indicated that hybridization and selection might produce plants with increased seed sucrose content.

The GCA effects for sucrose ranged from −7.4 to 7.0 for the F_2 analysis and from −7.2 to 10.9 for the F_3 analysis. The greatest negative GCA effects, i.e. contribution to lower sucrose, were exhibited by 'Late Giant' followed by 'Tomahomare' in the F_2 analysis and PI 379621 in the F_3 analysis followed by 'Late Giant'. The parents that consistently showed high GCA effects in both F_2 and F_3 analyses were 'Kanrich', 'Pella', 'Verde' and V81-1603. Others that showed positive GCA effects in F_2 but negative GCA effects in the F_3 were PI 506852, PI 399055 and VS95-50. These results suggested that these parents were not stable in performance over generations. The GYI was significant and might have played a role in the performance of these parents. Parents that showed negative GCA effects in both F_2 and F_3 analyses were 'Tomahomare', PI 379621 and 'Late Giant'.

The high sucrose content of certain crosses ('Pella' × 'Verde', 'Pella' × VS95-50, 'Verde' × PI 399055, 'Tomahomare' × PI 506852, 'Tomahomare' × VS95-50, PI 506852 × PI 399055, PI 506852 × 'Late Giant' and PI 379621 × V81-1603) showed strong SCA effects in both the F_2 and F_3 analyses. These crosses were higher than the overall means in sucrose content, because in most cases these crosses contained at least one good combining parent, indicating that such combinations should yield desirable transgressive segregants. In a study of winged bean [*Psophocarpus tetragonolobus* (L.) D.C.] De Silva and Omran (1986) reported significant heterosis and transgressive segregation for number of seeds per pod. In our study significant reciprocal effects were also found among the progenies, ranging from −31.1 (PI 506852 × 'Tomahomare') to 37.1 ('Late Giant' × V81-1603). Some reciprocal crosses 'Verde' × 'Kanrich', PI 506852 × 'Kanrich', PI 399055 × PI 506852 and 'Late Giant' × V81-1603 showed positive and significant effects for sucrose in both F_2 and F_3 analyses. This indicated a strong GYI among parents for seed sucrose content.

Total sugar

The analysis of variance indicated that the GCA, SCA and RECIP mean squares were significant (P = 0.05). A value of 2.76 obtained for the ratio GCA/SCA in the F_2 analysis indicated that GCA was more important than SCA in determining the parents' hybrid performance. Similarly in the F_3 analysis, a value of 6.18 was estimated for the ratio GCA/ SCA suggesting that GCA was more important than SCA in determining hybrid performance (Table 1).

The parental mean varied from 67.6 to 115.1 mg/g with an overall mean of 95.0 mg/g in the F_2 analysis and varied from 61.9 to 115.1 mg/g in the F_3 with an overall mean of 81.4 mg/g (Table 2). In both F_2 and F_3 analyses 'Kanrich' showed significantly higher mean total sugar than the overall F_2 or F_3 means. In F_3 analysis, 'Pella', 'Verde' and V81-1603 had significantly higher means than the overall mean.

The crosses that produced significantly (P = 0.05) higher total sugar than the overall mean in the F_2 analysis were: 'Kanrich' × PI 506852, 'Verde' × PI 399055, PI 506852 × PI 399055, PI 506852 × VS95-50, PI 506852 × 'Late Giant', PI 379621 × PI 399055, PI 379621 × 'Late Giant', V81-1603 × PI 399055, V81-1603 × VS95-50, PI 399055 × 'Late Giant' and PI 399055 × VS95-50. The cross combinations with significantly higher means than the overall mean in F_3 analysis were 'Kanrich' × 'Pella', 'Kanrich' × 'Verde', 'Kanrich' × 'Tomahomare', 'Kanrich' × PI 506852, 'Kanrich' × PI 379621, 'Kanrich' × V81-1603, 'Kanrich' × PI 399055, 'Pella' × PI 379621, 'Pella' × V81-1603, 'Verde' × PI 399055 and V81-1603 × 'Late Giant'. In general, the majority of the crosses with higher mean values involved one parent having high GCA effects, indicating that additive × dominance gene action was involved.

The GCA effects are given in Table 2. Estimates of GCA effects for both F_2 and F_3 analyses ranged from −9.2 to 13.7. Parents with significantly lower GCA than zero in both F_2 and F_3 analyses were 'Tomahomare', PI 379621 and 'Late Giant' and those with significantly higher GCA than zero were 'Verde' and V81-1603. The estimates of GCA effects of parents indicated that 'Verde' and V81-1603 were good general combiners for high total sugar and may serve as genetic sources in breeding programmes for increased total sugar.

The cross combinations with desirable total sugar SCA effects in both F_2 and F_3 analyses were 'Pella' × VS95-50, 'Verde' × V81-1603, 'Tomahomare' × PI 506852, 'Tomahomare' × 'Late Giant' and PI 379621 × V81-1603. The RECIP effects were also significant and the cross combinations that showed high total sugar content in both F_2 and F_3 analyses were 'Verde' × 'Kanrich', 'Verde' × 'Pella', 'Tomahomare' × 'Kanrich', 'Tomahomare' × 'Verde', PI 506852 × 'Kanrich', V81-1603 × 'Kanrich', VS95-50 × PI 399055, 'Late Giant' × V81-1603 and 'Late Giant' × PI 399055. Reciprocal differences could be attributed to strong GYIs.

Correlations among the seed traits

The correlations between parental sucrose and total sugar mean values were positive and significant with values of 0.879** and 0.976**, in the F_2 and F_3 analyses, respectively. Similarly the parental GCA effects for sucrose were positively and significantly correlated (0.855** for F_2 and 0.879** for F_3) with total sugar. These correlations suggest that sucrose and total sugar are strongly interrelated and selection for increased sucrose should result in increased total sugar.

Discussion

In this study, high genetic variability and low environmental influence were found for both sucrose and total sugar seed traits. However, the environmental component might have been underestimated as the experiment was conducted in a single location over two growing seasons.

In both sucrose and total sugar seed traits, the F_3 GCA/SCA ratios were higher than the F_2 ratios by more than twofold. In the F_3 analysis, the dominance component of genetic variance should theoretically, be reduced by half compared with the F_2 analysis. Thus a reduction was expected in the relative magnitude of SCA mean squares in the F_3 (Nienhuis and Singh 1986). In the F_3 analysis, general combining ability was more important in predicting the parents' contribution to the progenies.

As indicated, the progeny means for sucrose and total sugar differ from F_2 to F_3 generations. This suggested that parental performance was not stable over growing seasons. These results were supported by the significant GYI observed among the parental lines. The parent lines with consistently significant higher sucrose and total sugar content in both F_2 and F_3 progenies were large-seeded vegetable types (Mebrahtu et al. 1991). The diversity among the parents for sucrose and total sugar content indicated that hybridization and selection might produce plants with increased seed sucrose and total sugar content. The components responsible for sweetness of raw vegetable soybean seeds are free alanine and sucrose (Masuda 1991). Thus, sucrose level in vegetable soybean seeds retail is an important factor.

In general, the majority of the crosses that showed significant and negative SCA effects involved either one or both parents having low GCA effects indicating additive or additive × dominance gene action. Because non-additive variances were significant, the breeders' goal should be to achieve homozygosity rapidly while reserving intense selection pressure for later generations. Consequently, the 'Single seed descent' breeding system (Brim 1966) would be most relevant, as it could advance the population to homozygosity rapidly by advancing two to three generations a year with minimal selection prior to F_5 or F_6. In the F_5 or F_6 generations, extracting superior lines from advanced populations could be accomplished by selection across environments. Since the plant or line performance would be repeatable and unbiased in the subsequent generation, the superior lines isolated could then be tested across environments to minimize the bias caused by genotype × environment interactions which were large for these traits.

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